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CHAPTER 11

SUMMARIZING DISCUSSION AND FUTURE PERSPECTIVES

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Over the last 13 years, from 2006 until 2020, approximately 200.000 multiple sclerosis patients have been treated with natalizumab in 80 countries. The major advantage of natalizumab treatment in patients with relapsing remitting multiple sclerosis (RRMS) is its high efficacy with a drastic reduction of relapses and MRI activity.[1, 2] Its major drawback is the increased risk of progressive multifocal leukoencephalopathy (PML), a possibly lethal brain infection caused by the John Cunningham virus (JCV).[3] Other disadvantages of natalizumab treatment include the intravenous administration resulting in frequent hospital visits and relatively high medication costs (>€1700,- per 300mg infusion based on Dutch healthcare costs). Besides PML, the safety profile of natalizumab is favorable and the drug is well tolerated.[2] Furthermore, patients report an improvement of health related quality of life after initiation of natalizumab.[4] With over a decade of experience, natalizumab still holds a solid place in the treatment of RRMS, despite the exponential increase of newly developed immunomodulatory agents.

Research has led to the deeper understanding of the different effects of natalizumab which reaches further than the immune system only.[5, 6] There have been numerous observational trials reporting on long-term efficacy[7, 8] and patients wellbeing under natalizumab treatment.[4] Furthermore, PML risk estimation tools and monitoring guidelines have been developed and improved.[3, 9]

Should we take more drastic measures in trying to increase drug convenience for patients and to lower costs, all while retaining optimal drug efficacy? Can we even begin to desire to actively decrease PML risk when we have not fully elucidated the pathophysiology of natalizumab associated PML? And could it be so simple that personalized extended interval dosing of natalizumab is the answer to all of these unmet needs?

In this chapter we will discuss biomarkers which could guide personalized natalizumab treatment. We will review personalized natalizumab treatment with possible advantages and disadvantages in comparison to standard interval and extended interval treatments. This will also be discussed in light of PML risk and possible PML biomarkers. Finally, and perhaps most excitingly, we will discuss the future perspectives regarding natalizumab

treatment and elaborate on our ideas for improving natalizumab treatment in current practice with benefits for patients and society.

A biomarker to guide personalized natalizumab dosing

Natalizumab is administered intravenously. Free natalizumab attaches to CD49d (α4 subunit) of VLA4 (α4β1 integrin) on lymphocytes. Lymphocytes with natalizumab bound VLA4 are prevented to binding to VCAM1 expressed on endothelial cells and therefore cannot migrate over the blood brain barrier into the central nervous system (CNS). However, it would be over simplistic to assume that all free natalizumab immediately attaches to VLA4 where after natalizumab is cleared in a linear fashion. More realistically, a complex interplay exists between free natalizumab, VLA4-bound natalizumab, VLA4 expression and endogenous IgG4. Furthermore, individual patient characteristics can influence pharmacokinetic and pharmacodynamic values.[10]

Thus, with multiple factors influencing pharmacokinetic and pharmacodynamic parameters, what would be the most suitable biomarker to guide personalized natalizumab treatment?

We studied serum natalizumab concentration as a potential biomarker. The assay for measurement of serum natalizumab that was used in our research was developed at Sanquin Laboratory, Amsterdam. In short, a cross-linking assay using polyclonal rabbit anti-natalizumab F(ab)2 fragments and a mouse anti-IgG4 monoclonal antibody for detection was used.[11] In **chapter 3** we tested natalizumab trough concentrations in 80 RRMS patients treated with natalizumab in the standard 4 week interval. One of the most striking findings of this study was the inter-individual variability of trough concentrations within a set dose and treatment regimen. Natalizumab trough concentrations ranged from 0.1 to 80.0μg/ml. It is of great interest to try understand this variability as the large majority of patients has high trough concentrations, indicating a certain level of overtreatment. In our study, we found the concentration to be inversely associated with body weight. This finding was confirmed by others.[10, 12, 13] Muralidharan et al. performed a large pharmacokinetic and pharmacodynamic study evaluating natalizumab concentration and influencing factors. In their report, natalizumab trough concentrations in patients weighing 100kg was approximately 40% lower in comparison to patients weighing 60kg. However, they found body weight to be only a minor factor (6%) in explaining the variability of natalizumab

trough concentrations, comparable to the findings of our study. Foley et al. found body weight to explain a higher part of the variability of natalizumab trough concentrations, approximately one third.[13] Still, body weight seems an insufficient marker to accurately predict natalizumab trough concentrations and body weight based individualized natalizumab treatment is likely not the optimal approach, even though some have suggested this.[12] We found that age, sex and treatment duration were not associated with natalizumab trough concentrations which is in agreement with some other studies.[10, 14] However, in contrast to our findings a recent report showed that age and treatment duration had a moderate influence on natalizumab trough concentrations with concentrations slightly increasing over time.[13] This could be explained by the fact that that study included older patients (25% of age between 57-76 years) and the fact that drug metabolism can decline in older patients. Reviewing ours and other pharmacokinetic studies on natalizumab, the variability of natalizumab trough concentration stays largely unexplained, with unknown factors contributing to clearance of natalizumab. As inter-individual natalizumab trough concentrations vary widely, we studied the intra-individual variability by measuring two consecutive samples. The intra-individual variability or the trough concentrations appeared small, with larger variation when the trough concentration exceeded 30µg/ml. The stability of intra-individual natalizumab trough concentrations has been studied in eleven patients in another study where longitudinal intra-individual pre- and post-infusion natalizumab concentrations were comparable.[14] It would be of value to confirm this in larger studies.

Another finding of our study described in chapter 3 is the lack of association of natalizumab concentration with disease activity. In our study, a minority of patients (17.7%) experienced disease activity (radiological or clinical) over a median follow-up of 5.1 years and trough concentrations were comparable to patients without disease activity. A different study has reported an increase of disease activity with lower natalizumab concentrations,[15] but the cut-off concentration for disease activity was 1µg/ml. In our study all but one patient had adequate trough concentrations of >2µg/ml explaining the fact that we did not find an association of natalizumab concentration and disease activity. The one patient in our study with very low natalizumab trough concentrations appeared to be having persistent high anti-natalizumab antibodies. This case is described in detail in **chapter 4.** Remarkably, this patient had persisting high titer antibodies during 8 years of natalizumab treatment. Transient anti-natalizumab antibodies are quite prevalent with up to 58% of patients

having antibodies during the first year of treatment.[15] Persisting antibodies are reported in 3.5-9.4%,[15-18] but different definitions of persistence of antibodies have been used. Anti-natalizumab antibodies are inversely associated with the natalizumab concentration,[15] and persisting antibodies are logically associated with continuation or recurrence of disease activity under therapy.[16] Because of this, we were surprised that the patient had not experienced any disease activity since starting natalizumab therapy despite having experienced disease activity prior to the initiation of natalizumab. Longitudinal pharmacodynamic and pharmacokinetic measurements showed a drop to sub-therapeutic levels after 7 days of infusion, due to the very high titer of antibodies. Another report shows that clearance of natalizumab is 2.5 fold higher for patients with anti-natalizumab antibodies in comparison to patients without antibodies.[10] If we were merely surprised about the absence of disease activity despite high titer antibodies, we were astonished by the fact that the patient experienced recurrence of disease activity after discontinuation of natalizumab. Obviously, no causality can be proven with this one case, but it has broadened our view with the consideration that certain patients might need minimal effects of natalizumab for adequate disease suppression. In patients without anti-natalizumab antibodies, return of disease activity has been reported after approximately 12 weeks of withdrawal of natalizumab when natalizumab concentrations drop below 1µg/ml.[19] As natalizumab trough concentrations vary widely between patients four weeks after the last infusion, we hypothesized that the moment of recurrence of disease activity after discontinuation of treatment could be predicted by natalizumab trough concentrations. In **chapter 5** seventeen patients with twenty-two pregnancy related discontinuations of natalizumab were described. Disease activity and confirmed disability progression were associated with longer time to conception.[20, 21] All patients were on the standard four week interval prior to natalizumab discontinuation. Natalizumab trough concentration was measured in 21 cases before treatment discontinuation, again with a large variability of trough concentrations ranging from 3 to 120µg/ml. Not confirming our hypothesis, time to relapse was not associated with natalizumab concentration. However, we only measured trough concentrations after a four week interval and for a more reliable prediction of time of recurrence of disease activity, a pharmacokinetic profile might be more valuable than a single measurement of concentration. It remains is notable that the earliest relapse in this study (2.5 months after treatment discontinuation) occurred in the patient with the lowest natalizumab trough concentration.

So how suitable is natalizumab concentration as a biomarker? In our opinion, this depends on the goal of use of the biomarker. The concentration seems insufficient to predict the time of (recurrence of) disease activity in an individual patient as the severity of the MS probably plays a more important role and this highly varies between RRMS patients. However, natalizumab trough concentrations >1-2µg/ml seem adequate to suppress disease activity and higher trough concentrations do not increase drug efficacy. Therefore this biomarker seems suitable in showing the excess of natalizumab which is still present in the large majority of patients after a four week interval. Furthermore, natalizumab concentrations can be easily measured by ELISA in fresh and frozen sera.

When measuring the natalizumab concentration, it is wise to realize its interactions and the fact that a (trough) concentration does not represent a complete therapeutic picture. The main mechanism of natalizumab efficacy results from the binding of natalizumab to VLA4. Bound natalizumab can be assessed on peripheral blood mononuclear cells (PBMCs). Natalizumab concentration is associated with the saturation of VLA4 with natalizumab.[10, 14, 22] We confirmed this in our studies described in chapter 6 and 7. A large pharmacodynamic study on natalizumab found a 50% VLA4 saturation to be associated with 2.5µg/ml free serum natalizumab and 80% VLA4 saturation to be associated with 10µg/ml.[10] Above a concentration of 10µg/ml, the saturation curve flattens and the excess of natalizumab is reflected solely by its concentration. Approximately five years ago, natalizumab receptor saturation of 70-80% was assumed to result in maximum drug efficacy,[23] however more recent studies show significant recurrence of disease activity only when the saturation drops below 20%.[19, 24] One should realize that the VLA4 saturation, as the natalizumab concentration, during a treatment cycle is always a dynamic process with maximum receptor saturations after infusion followed by a slow decline. Furthermore, VLA expression varies inter-individually,[25] per cell subset,[25] and VLA4 expression is decreased in natalizumab treated patients.[14, 25, 26] VLA4 expression in natalizumab treated patients is also a dynamic process as the expression is lowest after natalizumab infusion with a subsequent rise over the following weeks (in a 4 week interval).[14, 25] Thus, VLA4 expression varies inter-individually, intra-individually per cell subset and at different time points depending on timing of treatment, complicating the potential use of VLA4 receptor saturation as a biomarker. Furthermore, regarding different levels of VLA4 expression, a percentage of bound VLA4 may not be as appropriate as an absolute number

of open VLA4 as the latter might be more relevant in terms of binding VCAM1 in respect to migration of lymphocytes over the blood brain barrier. Future research will hopefully elucidate this process of open versus bound VLA4 in regard to the ability of different cell subsets migrating into the CNS.

To make matters just a bit more complicated, there is another factor influencing pharmacokinetics during natalizumab treatment. Endogenous IgG4, consisting of two isomers connected by hinge disulfide bonds, can exchange half-molecules, a process which is known as Fab-arm exchange.[27] Natalizumab, a humanized IgG4 antibody, exchanges Fab-arms with endogenous IgG4.[28] Depending on natalizumab concentrations and levels of endogenous IgG4, Fab-arm exchange will occur which results in partly monovalent natalizumab (half natalizumab, half endogenous IgG4).[11] Monovalent natalizumab will form a weaker bond with VLA4.[11]

Personalized dosing of natalizumab

Multiple sclerosis, and especially RRMS, is a highly heterogeneous disease. Disease severity can extend from one extreme to another. Making a right prognosis in the beginning of the disease is important to advise the patient about treatment and lifestyle decisions. Baseline clinical and radiological characteristics, environmental and demographic factors and biomarkers are all prognostic factors to be considered. Furthermore, patient related factors such as comorbidities, family planning and preferences including drug convenience, safety and side effects should be taken into account. All these different prognostic factors and patients preferences should guide the physician in a personalized treatment of multiple sclerosis.[29] This personalized approach regarding treatment decisions is gaining ground, but after choosing wisely, all disease modifying treatments (DMTs) are prescribed in a set dose and treatment regimen following FDA and EMA advise. This is expected as most of the current DMTs are not suited for personalized dosing. However, in the case of natalizumab, therapeutic drug monitoring is an option in which the medication dose is based on pharmacokinetic values, usually the drug concentration in the blood.[30] The aim is to personalize the dose and treatment regimen according to the pharmacokinetic characteristics of the patient resulting in a decrease of medication and treatment burden and possibly complications.

In **chapter 6** we report on outcomes of the PDNMS trial; a prospective single-arm multicenter trial regarding personalized dosing of natalizumab in MS. We

extended the infusion interval based on natalizumab trough concentrations in serum. Only patients without disease activity (defined as the lack of relapses and active T2 lesions) in the year prior to the study were included. Personalized extended interval dosing did not lead to disease activity during one year follow-up or during the extension phase. To our knowledge, we are the first to personalize natalizumab treatment intervals in RRMS patients and to apply therapeutic drug monitoring in a DMT for the treatment of multiple sclerosis. However, extended interval dosing of natalizumab has been reported and is of growing interest internationally in recent years.

The earliest study reporting on natalizumab extended interval dosing was published in 2014.[31] This retrospective study reports on 96 patients receiving a set six or eight week infusion interval for a minimum of six months. Disease activity (relapses and MRI activity) were comparable between patients on extended interval dosing and standard interval dosing with a mean follow-up of 20 and 22 months respectively. A selection bias could influence the results as physicians could easier decide to extend intervals in patients with milder and non-active disease. The largest study reporting on disease activity when applying natalizumab extended interval dosing included 905 patients on extended interval dosing, defined as three or more consecutive extended infusions with an interval ranging from four weeks and three days to eight weeks and five days.[32] In this retrospective chart review, extended interval dosing patients were compared to 1099 patient on standard interval dosing with comparable disease activity. Selection bias, incomplete and non-universal outcome measures and poorly described duration of follow-up limit interpretation of these results. However, this study does reflect the growing trend of experimental extended interval dosing of natalizumab, at least in the United States, indicated by the high number of patients in this cohort. The most recent study reporting on natalizumab extended interval dosing retrospectively studied disease activity in patients on extended dosing defined as an infusion interval of five to eight weeks for three months or longer.[33] During a mean follow-up of 11.8 months, disease activity was comparable in 85 patients during extended interval dosing compared to earlier standard interval dosing. More retrospective experiences with extended interval dosing have recently been presented with comparable results.[34, 35] Recently, Biogen, the manufacturer of natalizumab, has started a large international prospective randomized trial (NOVA) studying a six week interval versus the standard four week interval.[36] This study will run until 2021 and results are expected in 2022.

When reviewing the studies so far, it is difficult to draw conclusions on the true efficacy of natalizumab in extended interval dosing as different intervals are studied in retrospective settings. In relation to our study, it is of importance to discuss the advantages and disadvantages of extended interval dosing versus personalized extended interval dosing. Personalized dosing asks more expertise of the treating physician with measuring natalizumab concentrations and adjusting the interval accordingly. In our study we longitudinally measured natalizumab concentrations and adjusted the interval until patients reached a trough concentration of approximately 10µg/ml. However, the final interval was accurately predicted by the baseline trough concentrations in a four week interval, so testing the concentration before every infusion seems unnecessary. Still, as we saw one patient in our study with unexpected low natalizumab trough concentration after 1.5 years of personalized dosing, regular low frequently testing of the natalizumab trough concentration seems necessary after initiating personalized interval dosing of natalizumab.

Personalized dosing by therapeutic drug monitoring has the advantage of addressing a patient's biological needs with the options of a four to eight week interval. A set extended interval of six to eight weeks might induce disease activity in a minority (5-10%) of patients with low (<10µg/ml) natalizumab trough concentrations in a four week interval. For populations with higher body weights this risk is probably higher. In comparison to extended interval dosing, personalized dosing is probably playing it safe.

But how safe should we aim for? In our study, we extended the treatment interval with one week when the natalizumab trough concentration exceeded 15µg/ml. We aimed for a trough concentration of approximately 10µg/ml as the saturation curve flattens above this level of free natalizumab. However, as stated above, we expect recurrence of disease activity when the concentrations drop below 1µg/ml or below 20% receptor saturation. An aim of 10µg/ml with a corresponding 80% saturation might be playing it too safe and further extension of the intervals to an aim of 2-5µg/ml trough concentration could possibly be a superior approach with further decreasing costs and hospital visits with continued optimal drug efficacy.

In our study of personalized extended interval dosing, the large majority of patients chose to remain on their personalized interval after one year follow-up, which seems quite logical as hospital visits decrease. However,

during inclusion of the study many patients did not desire participation in personalized dosing as they 'felt they were low on drug concentrations' at the end of the four weekly treatment cycle. This feeling resulted from an increase of MS symptoms. This natalizumab wearing-off effect is recognized by most MS neurologists, but literature and understanding regarding this phenomenon is limited. In light of the increasing trend of (personalized) extended interval dosing, we studied this wearing-off effect in **chapter 7**. Our main research question was if the wearing-off effect truly arises due to a low natalizumab state reflected by low concentrations and or receptor saturations. We found similar natalizumab concentrations and receptor saturations in patients experiencing a wearing-off effect and patients without a wearing-off effect. Interestingly, our cohort included patients on standard (n=62) and personalized extended (n=31) treatment intervals. The wearing-off effect was reported in eleven patients on personalized extended interval dosing, of which four patients reported a decrease, one patient reported an increase and five patients reported no difference of the wearing-off effect after initiation of personalized dosing compared to prior standard interval dosing. Unfortunately, these numbers are too low to draw conclusions, but our study does not show any evidence in the direction of an increase of wearing-off effect after extending intervals. Furthermore, our study shows the wearing-off effect does not reflect a low pharmacokinetic/dynamic state of natalizumab. As the wearing-off effect is highly prevalent in our and other reports[37-39] with a prevalence of 54-67%, it is important to explain to patients that this effect is not a sign of imminent disease activity and likely will not increase with (personalized) extended interval dosing.

PML pathophysiology and new ways for risk reduction

The major disadvantage of natalizumab treatment in RRMS is the increased risk of PML. As of September 2019, 825 cases of PML have been reported with association to natalizumab therapy (personal communication Biogen). Risk factors have been identified over the last decade and this has led to risk monitoring tools[3] which can direct the physician and patient to a substantiated decision regarding (dis)continuation of treatment with natalizumab when JCV positive. Risk estimation is very important in respect to PML, however, reducing the risk would be even more valuable.

So far, there are no guidelines on how to abate PML risk in natalizumab treated patients with the exception of discontinuation of treatment. When considering how to decrease the risk, there are various options of intervention.

First, we can try influence the risk of JCV conversion from negative to positive, secondly we can try influence PML pathophysiology and thirdly we can try finding a treatment when PML does occur.

The JC virus is inhaled or ingested by humans after which it is latently present in kidneys and bone marrow.[9] Just over half of the population is JCV positive and an annual seroconversion of 1-2% is assumed.[40] The rate of JCV seroconversion under natalizumab treated patients is substantially higher, with annual conversion rates of 4-36%.[41] As the risk of PML is almost negligible in JCV negative patients treated with natalizumab, it is important to understand this increase of seroconversion. In **chapter 8** we investigated JCV status and seroconversion in natalizumab treated RRMS patients in relation to natalizumab concentrations. We found no association of JCV status or conversion with natalizumab trough concentrations. This implies that extending the intervals to lower trough concentrations would not affect the JCV seroconversion. However, in a meta-analysis regarding JCV seroconversion, the conversion rate from a large dataset from Salt Lake City with many patients on extended interval dosing was lower than the conversion rates from a large dataset from Germany where most patients received standard interval dosing.[41] Others have also studied possible relations with the risk of JCV seroconversion. Achiron et al. found transcriptional differences between seroconverters and non-seroconverters in natalizumab treated patients. Furthermore, they found an over-expression of genes that are associated with viral entry into host cells in patients who seroconverted during natalizumab treatment.[42] Unfortunately, this does not lead to a possible route for intervention of the PML risk.

So far, natalizumab extended interval dosing has been designated as the only intervention to possibly lower the risk of PML in natalizumab treated patients.[31-33] The rationale behind this is to decrease natalizumab levels at the end of the cycle to increase immune surveillance in the CNS and induce JCV suppression, simultaneously keeping natalizumab levels high enough to halt MS disease activity. In light of this hypothesis, we retrospectively tested longitudinal natalizumab trough concentrations in five patients with natalizumab associated PML which is described in **chapter 9**. We hypothesized that PML patients might have higher trough concentrations than matched controls. However, we did not find higher concentrations in five patients developing PML in comparison to the controls. Moreover, three of the five patients had lower natalizumab trough concentrations (10-15µg/ml) than

the mean of 25µg/ml we described in chapter 3. Even though this is a case series where no conclusions can be made regarding PML risk, our findings imply that a trough concentration of 10µg/ml is not protective of developing PML. When considering the natalizumab receptor saturation as a risk factor for PML, a trough concentration of 10µg/ml resulting in approximately 80% saturation[10] could still be too high. In light of personalized extended interval dosing with an aim of 10µg/ml, playing it too safe in regards of disease activity might result in playing it unsafe regarding PML risk.

All these speculations about a reduction of PML risk with less natalizumab exposure relied solely on hypotheses, until very recently it was confirmed that patients on extended interval dosing indeed have much lower risk on developing PML.[43] In the USA, the TOUCH registry prescribing program is a mandatory US risk evaluation and mitigation strategy for physicians prescribing natalizumab for MS. This registry represents a large database on natalizumab patients on standard and extended interval dosing and occurrence of PML. Zhovtis Ryerson et al. compared natalizumab associated PML in JCV positive patients treated with standard interval dosing and extended interval dosing. The cut-off for extended interval dosing was ≤10 infusions per year (≥5.2 weeks interval). Three categories were formed: extended interval dosing in the last 18 months after standard interval dosing, extended interval dosing during >6 months in between standard interval dosing, and primarily and only extended interval dosing. In all three categories, most in the latter category, there was a drastic PML reduction in extended interval dosing compared to standard interval dosing. Unfortunately, no pharmacokinetic of pharmacodynamic measurements are reported. We can however speculate that natalizumab trough concentrations in this cohort were probably lower than 10µg/ml as the definition of extended interval dosing was ≤10 infusions per year, which likely resulted in most patients on a set six weeks interval. In chapter 6 we showed that patients with a trough concentration of 30-45µg/ml in a standard interval dropped to the aim of 10µg/ml with a six weeks interval. Taking a mean natalizumab trough concentration of 25µg/ml after a standard interval (chapter 3), extending the interval to six weeks will result in trough concentrations of below 10µg/ml. Thus, extending interval dosing seems protective of PML with likely lower (<10µg/ml) natalizumab trough concentrations. The study of Zhovtis Ryerson et al. did not evaluate disease activity and it is quite plausible that extending interval dosing in patients with low natalizumab concentrations resulted in an increase of disease activity. That being said, we are hopeful there exists a cut-off in natalizumab trough

concentration resulting in PML protection and adequate MS suppression, but such a cut-off is yet to be defined.

It is however unlikely that the risk of PML can be fully obliterated by extended interval dosing and cases of PML have been described in patients on extended intervals.[44] Natalizumab associated PML has a mortality rate of 24% and older age, high pre-PML disability, high CSF JCV copies, multifocal damage on MRI and higher Karnofsky Performance Scores are predictors for a poor outcome.[45] When PML occurs, rapid immune reconstitution is necessary to battle the infection and natalizumab should be discontinued immediately when a diagnosis of PML is considered. As natalizumab can be detectable in blood up to 200 days after the last infusion,[46] some have advised a rapid removal of natalizumab by plasmapheresis to accelerate immune reconstitution. However, the effect of plasmapheresis on survival or clinical outcome of natalizumab associated PML is unclear.[47] Based on in vitro efficacy, mefloquine is widely used in the treatment of PML, but clinical evidence is largely missing.[48] Next to mefloquine, mirtazapine is usually prescribed in the treatment of natalizumab associated PML with a possible benefit in clinical outcome.[49] Other therapies such as maraviroc and filgrastim have been described in cases with natalizumab associated PML.[50-52]

Unfortunately, an effective treatment for natalizumab associated PML is currently lacking. When a patient is diagnosed with PML, the patient is clinically and radiologically monitored. Frequent MRI's are performed for estimation of PML progression and to monitor a possible immune reconstitution inflammatory syndrome (IRIS), a condition which is frequently treated with steroids. However, frequent MRI's are expensive and stressful for the patient and a need exists for easily available PML biomarkers. Neurofilament light is a biomarker with increasing use in various neurological diseases.[53] Neurofilament light is a neuronal cytoplasmic protein and is abundant in large myelinated axons. In axonal loss due to a variety of reasons, an increase of neurofilament light can be found in the cerebrospinal fluid (CSF).[53] Fortunately, there is a strong correlation of neurofilament light in CSF and serum,[54] making it a more useable biomarker. Neurofilament light is associated with disease activity in MS.[54] Furthermore, serum neurofilament light was shown to be increased in natalizumab associated PML.[55] To confirm this finding, we longitudinally tested serum neurofilament light in five patients with natalizumab associated PML in **chapter 10**. In four out of five patients serum neurofilament light increased prior to PML diagnosis. PML lesions

were identified by raters who scored previous scans for MS lesions. The PML lesions were manually delineated by three raters and PML lesion volume was calculated, a method that is further described elsewhere.[56] We found an exceptionally strong correlation between serum neurofilament light and PML lesion volume, indicating the potential of serum neurofilament light as a biomarker in PML diagnosis and follow-up.

Future perspectives

In 2016, we started the PDNMS trial; personalized dosing of natalizumab in multiple sclerosis which is described in chapter 6. Despite this being not so long ago, at the start of the study we still assumed that 70-80% saturation was needed for optimal natalizumab efficacy and there were more non-believers than believers regarding the hypothesis that prolonging the interval could reduce PML risk. Within this tiny niche in MS healthcare much has happened over the past few years, and many studies have added knowledge about pharmacokinetic and pharmacodynamic values in regards to natalizumab efficacy. Furthermore, the first strategy for PML risk reduction has been described. All this valuable information provides the building blocks to start making real changes in optimizing natalizumab treatment. So let's proceed and aim for a global optimization of natalizumab treatment in patients with RRMS.

First things first. Should we personalize or extend? The NOVA study, initiated by Biogen, started in November 2018 with the aim of including 480 patients in 97 study locations.[36] The study randomizes natalizumab treated RRMS patients between a set four or six week interval. The study focusses primarily on disease activity but likely will bring us more knowledge about pharmacokinetic and pharmacodynamic values in relation to drug efficacy. We predict that a six weeks interval will be non-inferior to the standard interval in the large majority of patients (>95%) regarding drug efficacy. However, in the six week interval natalizumab trough concentrations will still vary widely between patients (although presumably less than within a four week interval), with some patient still receiving a relative overdose and some patients might still be more prone to PML than others. The NOVA study is not powered to make conclusions regarding PML risk as it will take thousands of patients for such a conclusion. Even though we feel this is a very valuable study and changing to a six weeks interval can bring major advantages, we feel that personalized dosing based on therapeutic drug monitoring is the way to proceed forward.

Within the Netherlands we are currently setting up a large national trial (the NEXT-MS study) to validate and implement personalized dosing of natalizumab in RRMS patients in the Netherlands. This study starts in 2019. Based on the results of the PDNMS trial, patients will receive a personalized interval after two natalizumab trough concentrations measurements in the standard four week interval. Below 15µg/ml patients will remain on the four week interval, between 15-30µg/ml patients will be put on a five week interval, between 30-45µg/ml patients will be put on a six week interval and above 45µg/ml patients will be put on a seven week interval. All patients will receive standard follow-up with an annual relapse assessment, disability scoring and MRI scan. Natalizumab trough concentrations will be measured after three and six months and every six months thereafter. However, as extensively discussed above, personalized dosing with an aim of 10µg/ml might not make the difference in regards of PML protection. Therefore, within this study a subgroup will receive personalized dosing with the an aim of approximately 5µg/ml natalizumab trough concentration. The design of this subgroup is comparable to the PDNMS trial. When our hypothesis proves to be right that optimal drug efficacy can also be reached with an aim of 5µg/ml, the entire study group will switch to an altered personalized schedule. Within the study, MS neurologists in the Netherlands will gain experience in natalizumab therapeutic drug monitoring and develop affinity with the meaning of natalizumab trough concentrations.

Despite lowering PML risk and increasing treatment convenience for the patient, personalized dosing results in substantial financial benefits for society. The medication costs of one infusion (300mg) are €1,754 and healthcare related cost per infusion are €1,645 ('Dutch Healthcare Authority'). Putting one patient on a 6 week interval instead of the standard 4 week interval, will result in an annual cost reduction of €14,729. As approximately 1000 MS patients in the Netherlands are currently treated with natalizumab, implementing personalized dosing could easily lead to an annual cost reduction of more than 10 million euros.

We will not limit our initiatives to the Netherlands. Within the timeframe of the NEXT-MS study, we will set up a network of international partners aiming to globally implement personalized dosing of natalizumab in RRMS. We should first ensure the logistics needed for therapeutic drug monitoring with comparable assays internationally. In the Netherlands, we use one validated assay[11] at Sanquin Laboratory which has excellent logistics worldwide.

Currently, we are internationally comparing our assay in measurement of natalizumab trough concentration. When logistics are set, an international study group will be formed to install study guidelines regarding personalized dosing of natalizumab. Most importantly, an international database must be developed to follow patients on personalized dosing with monitoring of the trough concentrations, disease activity and PML incidence. Then, we can make a final statement about the optimal aim for personalized dosing of natalizumab which hopefully will result in a drastic reduction of natalizumab associated PML, a firm decrease of the treatment burden of the patient, a significant reduction of natalizumab related costs, all while retaining optimal natalizumab efficacy.

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